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MODERNIZING COLOR AND VOLUME INDEX*

By CECELIA M. KORTUEM, R.N., M.T.†
Chicago, Illinois

Color Index represents the relative amount of hemoglobin contained in each red cell as compared to the amount of hemoglobin in a normal cell. It is the quotient of the percentage of hemoglobin divided by the number of red blood cells per cubic millimeter, expressed in percentage of the normal. The lack of absolute normals for color index has been confusing (1), (4).

A color index of 1 indicates that each red blood corpuscle contains the normal amount of hemoglobin. A *LOW* color index is characteristic of chlorosis. A *HIGH* color index is pathognomonic of pernicious anemia (1).

Hemoglobin reports have eliminated the percentage figure for some time, which in itself would discard the usual formula for obtaining the color index.

Observations are in accord with those of Todd and Sanford (4) namely; that variations in hemoglobin instruments, and the impossibility of fixing a normal standard for either red cells or hemoglobin applicable to all ages and to all localities, seem to make the color index calculations of little value. Some of these difficulties

* *Presented at the Annual Convention of the American Society of Medical Technologists, Philadelphia, June 8, 1942.*

†From the Laboratory of St. Vincent's Infant and Maternity Hospital, Dr. M. L. Blatt, Director and Chief of Staff.

BMT

were surmounted by Wintrobe (3) who, devised a formula for obtaining the mean cell hemoglobin concentration. His formula is the hemoglobin in grams per 100 cc divided by the volume of packed cells per 100 cc of blood, times 100. This quotient can be employed in a simple formula for obtaining the color index unit.

A certain percentage of cells are observed with insufficient hemoglobin and a certain percentage with sufficiently large amount of hemoglobin to be hyperchromic. A cell hemoglobin concentration curve reveals the percentage in each group, denoting borderline cases and progress in the event of therapy response.

The hemoglobin content and size of the erythrocytes has not aroused as much interest in clinical medicine as its importance demands. This is probably because of technical difficulties encountered in determinations and a lack of absolute standards (2). The lack of absolute normals for Volume Index has also proved very confusing.

Volume Index, is a term introduced by Capps (4) to express the average size of a red cell compared with the accepted normal. It is the quotient obtained by dividing the volume of packed red cells by the number of red corpuscles, each expressed in percentage of the normal (4). Todd and Sanford (4) and a number of other writers state that the Volume Index is a simple and satisfactory means of expressing the average size of the red cells, and that it gives accurate information more readily than does measuring them with the micrometer eyepiece.

Kato (5) states: "It must be remembered, that the measurement of cell volume is not identical with the estimation of cell diameter, for these are two different attributes of a blood cell. Undoubtedly in most instances the hematocrit method yields the essential data, but there are many other conditions in which the measurement of cell diameter is important for an accurate diagnosis and prognosis."

The simplest and most reliable method of obtaining the mean cell diameter is by direct hemocytometry. The Price-Jones (6) method of outlining the image of the cell is time-consuming and difficult. The use of a simple hemo-protractor (7), has been found convenient and easy to use. It not only gives the curve for cell diameter, but it can also be used to calculate the hemoglobin concentration of

the cells. Furthermore, this means of deriving the mean cell hemoglobin concentration when the mean cell diameter ranges from 7.0—7.8 microns, parallels the method formulated by Wintrobe (3); but, when the mean cell diameter is less than 7.0 or more than 7.8 microns, this method surpasses the results obtained by Wintrobe's formula. The greater the figure for the volume of packed cells dividing the grams of hemoglobin, which is Wintrobe's formula, the smaller the figure for the percentage of cell hemoglobin concentration; the lesser the figure for volume of packed cells, the higher the percentage of cell hemoglobin concentration. In either event the report would be misleading if there is abnormality of cell diameter. Two years ago, I devised a correction factor table for cell diameter (7), which can be used to correct for mean cell hemoglobin concentration in case of cell size abnormalities.

A hematological report of 36.0% mean cell hemoglobin concentration with a mean cell diameter of 6.37 microns would facilitate diagnosis and prognosis, whereas, the same figures before applying the cell diameter correction factor would be 30.4% mean cell hemoglobin concentration. With or without estimation of mean cell diameter this latter figure would denote insufficient hemoglobin in the cells, when actually the cells contain more than enough hemoglobin for their size.

Procedure for measuring the cell diameter and center pale area of the erythrocytes:

A hemo-protractor is drawn to scale according to the magnification of the microscope to be used. Due to variations in the magnification of microscopes it is not practicable to devise a universally applicable hemo-protractor.

To devise a hemo-protractor: Attach a Camera Lucida having a 3-4 inch arm length for holding the reflecting mirror, to the top of the monocular tube of the microscope, adjust the mirror at a 45° angle (Plate 1). Using the oil immersion objective and a drop of oil on the haemocytometer chamber, locate a 1/16 square; this has a 50 micron magnified line on each side of the square. (Figure 1) Draw the reflection of this square on a piece of paper and measure with a millimeter scale to determine the exact magnification. Using this same scale the graduated scale is drawn in with India ink and

a fine point pen. The details are filled in to suit your need and artistic desire.

To measure, the cell image is projected onto the surface of the protractor, which is moved freely on the table until the cell fits snugly between the lines on the scale and the diameter read direct as marked on the scale. While measuring the periphery of the cell, move the scale until the diameter of the center pale area fits equally snugly on the scale and tally each measurement. (Figure 2) From the work sheet a curve may be plotted in addition to providing a permanent record for the files.

The blood smears used for these determinations are those which are routinely made for the differential leucocyte counts. The film should be carefully spread so as to avoid aggregation of the cells, which would obscure the clear image. A thin evenly distributed film of blood on a slide, should be the pride and joy of every laboratory technician. The stains of Romanowski are very satisfactory.

Tally the periphery of 100 cells of a blood smear, made as described, together with the diameter measurements of the center pale area of the cell. (Table 1) To calculate the mean cell hemoglobin measurement subtract the mean diameter of the center pale area from the mean diameter of the cell. The normal average diameter in Chicago region is 7.5 microns with 2.5 microns (33.3%) of center pale diameter and 5 microns, 2½ microns (33.3%) on either side of the pale center, of hemoglobin.

The standard deviation, the coefficient of variation, the difference between the two means, based upon 65,000 measurements (100 cells in 650 slides), prove that there is no difference between this method of obtaining the percentage of mean cell hemoglobin concentration, and the determinations by the hematocrit method and Wintrobe's formula (3). Hematocrit determinations can be very misleading if the anticoagulant is not in exact dilution and ratio to the red blood cells to prevent their distortion (8), (9).

To report color index in units, divide the percentage of the mean cell hemoglobin concentration (CCH%) by the normal percentage of mean cell hemoglobin concentration (N/CCH%). (Figure 3). This formula has been found practical and accurate. It can be applied if the mean cell hemoglobin concentration is derived by direct hemocytometry (5), (6) or by Wintrobe's formula.

In a previous paragraph the question was brought up regarding the exact amount of hemoglobin a cell could contain before the picture would be hyper or hypochromatic. Cells of 456 slides of children's blood were measured and tabulated according to their mean cell hemoglobin concentration. These cells were within the volume range of 1 or an average cell diameter of 7.0—7.8 microns. The color index is also tabulated from the formula of the percentage of the mean cell hemoglobin concentration over the normal. (Table 2) Group A, shows figures that denote hypochromasis. Group B, shows figures considered normal. Group C, shows figures denoting hyperchromasia.

It was observed that 207 slides from cases, antepartum, ranged in mean cell hemoglobin concentration from 30.0% to 38.3% with the mean at 34.6%. The color index unit for this group averaged 1.02.

Summary—Percentage of hemoglobin, ordinarily used to calculate color index has been eliminated. A simple formula is presented for determining the units of color index by using the percentage of the mean cell hemoglobin over the normal.

To modernize the color index is to report the percentage of the mean cell hemoglobin concentration. To modernize the volume index is to report the mean cell diameter in microns.

It is recommended that the terms color and volume index be discarded and the report of actual figures be used for hematological reports.

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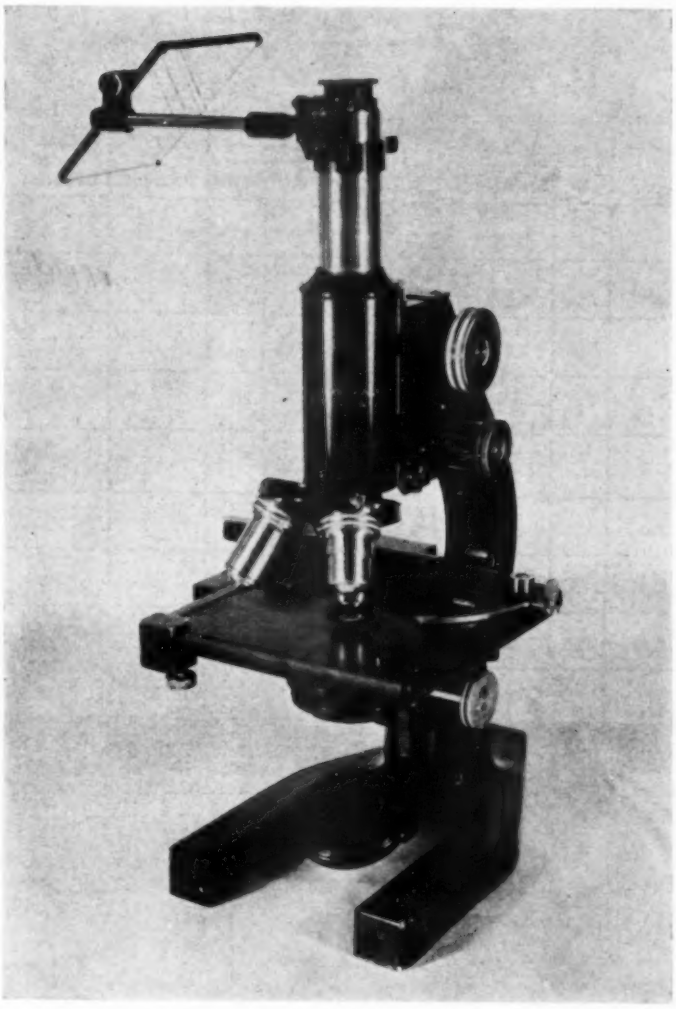
PLATE 1



Photograph of a microscope with camera lucida attached to its monocular tube.

TABLE I

Diameter in Microns	Center Pale Area of Cells							Cells Counted	Cell Total Diameters
	.05	1.0	1.5	2.0	2.5	3.0	3.5		
5.5									
6.0			11					2	12.0
6.5		11		////	11	11		11	71.5
7.0	1	////	11	111	////	////		21	147.0
7.5		111	11	11	//// //// ////	////		27	202.5
8.0	111		////	111 ////	//// 1	////	1111 ////	36	288.0
8.5				111				3	25.5
Total	2.0	10.0	16.5	42.0	70.0	51.0	31.5		
Grand Total	223.0							100	746.5

Mean Cell Diameter..... 7.5

Mean Center Pale Area..... 2.2

Mean Cell Hgb 5.3

Mean C.C.H.% 35.3

Table 1. Distribution curve of cell diameters.

TABLE II

Group "A"										
#1—	0.00	0.00	0.00	0.00	0.65	0.70	0.75	0.80	0.85	0.90
#2—	00.0	00.0	00.0	00.0	21.6	23.3	26.0	26.6	28.3	30.0
#3—	0	0	0	0	0	4	1	1	5	28
#4—	0.0	0.0	0.0	0.0	0.0	0.8	0.2	0.2	1.1	6.0
		Total # 39					Total	9.3%		
Group "B"										
#1—	0.95	0.96	0.97	0.98	0.99	1.00	1.01	1.02	1.03	1.04
#2—	31.6	32.0	32.3	32.6	33.0	33.3	33.6	34.0	34.3	34.6
#3—	63	7	16	28	9	41	52	20	22	42
#4—	13.8	1.5	3.5	6.0	1.9	9.0	11.4	4.3	4.8	9.2
		Total # 300					Total	65.4%		
Group "C"										
#1—	1.05	1.06	1.07	1.08	1.09	1.10	1.15	1.20	0.00	0.00
#2—	35.0	35.3	35.6	36.0	36.3	36.6	38.3	40.0	00.0	00.0
#3—	8	12	35	13	20	18	18	3	0	0
#4—	1.8	2.6	5.4	2.7	4.3	3.9	3.9	0.6	0.0	0.0
		Total # 117					Total	25.5%		

Key: #1—Color index units; #2—Percentage of Mean Cell hemoglobin concentration; #3—Number of tabulations; #4—Percentage in each tabulation.

Table II. Tabulation of cells of 456 slides of children's blood. Group A, shows figures that denote hypochromasia. Group B, shows figures considered normal. Group C, shows figures denoting hyperchromasia.

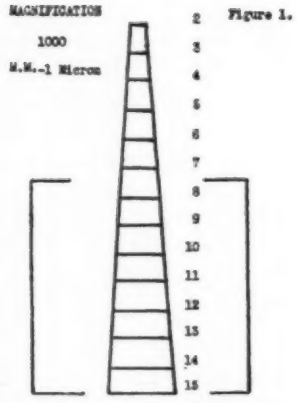


Figure 1. Hemo-Protractor constructed according to directions given in the text.

Diameters in Microns	Center Pale Area of Cells							Cells Counted	Cell Total Diameters
	0.5	1.0	1.5	2.0	2.5	3.0	3.5		
7.0									
7.5									
8.0									
8.5									
9.0									
9.5									
10.0									
Total									
Grand Total									

Mean Cell Diameter.....
Mean Cell Hgb.....
Mean Center Pale Area.....——
Mean C.H.C.% .....

Figure 2. Sample of work sheet for tallying cell diameters.

FIGURE 3

To Modernize the Color Index:

(A) $$\frac{\text{Mean cell hemoglobin concentration percentage}}{\text{Normal mean cell hemoglobin concentration percentage}}$$

Example: $\frac{39.9}{33.3}$ or 1.2 units of color index.

(B) $$\frac{\text{M C H C \%}}{\text{N/ M C H C \%}}$$

Example: $\frac{39.9}{33.3}$ or 1.2 C. I. Units.

Figure 3. A simple formula for obtaining the color index.

TWO THOUSAND STANDARD KAHN TESTS A DAY

By BETTINA B. CARTER

Kentucky State Board of Health, Louisville, Ky.

During a period of national crisis, a serologic laboratory is inevitably called upon to handle a greater volume of work than has been its lot in the past. The demands of selective service, added to those created by premarital and prenatal laws and the usual routine of diagnostic and treatment cases, create in the laboratory an increased need for organization and careful planning.

In the serologic laboratory of the State Board of Health of Kentucky, the technical staff has adjusted its services to handle two thousand standard Kahn tests during the period from eight o'clock in the morning until five o'clock at night. This is how the work is done.

The staff consists of eight individuals. One of those individuals handles the bulk of the spinal fluid examinations and contributes his assistance in the blood routine. Another person directs the details of administration, takes care of special work (quantitative and verification tests, antigen making, etc.) and cooperates in the routine. A third person is the laboratory helper who presides over the pipetting machine. The other five technicians carry the major portion of the routine blood examinations. The various phases of the work are distributed among the technical staff in order that each worker may have a certain variety in the day's procedure.

The actual performance of the tests is divided in four "runs", two in the morning and two in the afternoon. Preceding each run, every member of the group cooperates in unclotting and centrifuging blood specimens and, after the thirty minute water bath interval, in "culling" the specimens, i.e., examining each one for the presence of cells or other extraneous material, determining those serums which necessitate the performance of micro procedures, removing

badly hemolyzed or too chylous specimens. While this is being done, the antigen is mixed and an electric timer set for thirty minutes. When ten of those minutes are gone, the individual scheduled to pipette antigen takes the first two racks in a prepared series and adds antigen with the .5 cc pipette, then delivers to the back rows with a .25 cc pipette. These racks are passed to two workers who are prepared to pipette serum. The serum is added, the racks shaken by hand, placed in the shaking machine and the laboratory helper shakes them for the specified three minutes. The laboratory helper adds saline with the pipetting machine and passes the racks to the two individuals scheduled to read reactions. Results are read immediately by the aid of fluorescent lamps, recorded independently and comparisons made when the series is complete. After these comparisons are made, every positive and doubtful serum is again checked for the presence of any material which might obscure the reaction.

This arrangement requires five people besides the laboratory helper. The other two persons continue to prepare bloods, perform special tests and those on spinal fluid and, periodically, to mix antigen for the technician pipetting. Each "run" tests approximately 500 bloods. Sixteen suspension vials are prepared before each run, eight with 3 cc antigen each and eight with the appropriate amount of saline according to titer. These vials are stoppered with lead foil covered corks and kept until the appropriate time for mixing. The technician pipetting the antigen can plan exactly the times for mixing so that the pipetting for the entire five hundred tests goes on without a break and without ever exceeding the thirty minute restriction on the antigen suspension. The actual time for the run is about an hour.

The bloods are numbered consecutively, not for one day, but from the beginning of the year. They are placed in racks holding seventy-two tubes and each tube is labelled with the last three digits of its number, except for the first and last tubes in each rack, which bear the complete number. The Kahn racks have numbers from one to ten, a number painted in front of each tube space of the front row. Then the appropriate large number of the first of the ten serums to be tested in that rack is added in wax pencil when the racks are prepared for a run. Controls are set up for each antigen mixture.

The centrifuges have four trunnion carriers, each of which carries twenty-four 15 x 85 mm. tubes, the size used for the bloods. These carriers are numbered and are always filled in order and the serum poured in this order after centrifuging. The bloods are centrifuged for twenty minutes at 2000 rpm. At the end of that time, the serums can be poured off in the majority of instances.

Any reading discrepancies are checked by repeat tests and no reports are made without thorough checking of recorded results. Reports are not made on serums which are not absolutely clear. Reading of results is always handled by two individuals, observing and recording independently and then comparing their findings.

A tribute should be paid to the intelligence, loyalty and teamwork of the technicians who handle these specimens. Only through their devotion and ingenuity is the accurate performance of two thousand tests a day made possible.

THE DETECTION OF ACETONE IN URINE*

By PHYLLIS STANLEY, M.A., M.T.

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There is a definite relation between fat oxidation and carbohydrate metabolism. Under normal conditions the fatty acids are broken down forming acetoacetic acid which is finally oxidized to carbon dioxide and water. When the carbohydrate oxidation processes are impaired, the fatty acids are incompletely oxidized and the acetone bodies, namely: acetone, acetoacetic acid and B-hydroxybutyric acid are eliminated in the urine. Acetonuria may occur in a number of conditions where there is a disturbance in carbohydrate metabolism. Such diseases include diabetes mellitus, starvation, diggestive disturbances, pernicious vomiting of pregnancy, nephritis and chloroform or ether anaesthesia.

The amount of acetone present in the urine of the patient with diabetes mellitus is of greater importance than the amount of sugar present. Since the advent of insulin the frequency of acidosis in diabetics has been greatly reduced. Under normal conditions the daily output of acetone varies from 3 to 15 mgms. Under pathological conditions it can be increased to as much as 6 gms. per day.

The present conception is that the kidneys excrete diacetic acid which changes in the urine upon standing. Very fresh urine contains ten times as much diacetic acid as acetone. This fact is not important as both substances have the same significance clinically. Some of the chemical tests give the same reaction for either. The two may be differentiated by Gerhardt's ferric chloride test which produces a Bordeaux red color which disappears on heating when diacetic acid is present.

Chemical Tests for Acetone

A series of tests have been developed for the detection of acetone using undistilled urine and sodium nitroferricyanide with a

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color reaction in which there is a formation of ferropentacyanide with the isonitro compound of the ketone or the formation of such an ion with the isonitro-anine derivative of the ketone.

Some of the tests require that the distillate of the urine be used to overcome the reactions due to the presence of other substances in the urine which interfere or give similar results.

Method of preparing distillate:

1. 100cc. urine in distillation flask with side tube inserted into a test tube.
2. Add 3cc. acetic, phosphoric or hydrochloric acid.
3. Collect 35cc. of distillate.
4. Add 5 drops of 10% hydrochloric acid.
5. Redistil 20cc. and use for test.

Note: Rubber connections may give false positive reactions.

I. Lieben Iodoform Test
   1. 5cc. urine distillate in test tube.
   2. Render alkaline with potassium or sodium hydroxide.
   3. Add 1-2cc. Gram's iodine solution drop by drop.
   4. Allow to stand until a yellowish sediment of iodoform crystals is formed. The length of time that is required for these crystals to form varies with the amount of acetone. If very small amount of acetone is present, it may take 24 hours.
   5. Identify characteristic crystals.

This test is not as accurate as Gunning's test since alcohol and aldehyde yield iodoform. Since urines from patients in the advanced stages of diabetes contain alcohol this test is not desirable.

II. Gunning Iodoform Test
   1. 5cc. urine distillate in test tube.
   2. 5 drops Lugol's solution.
   3. 5 drops ammonium hydroxide.
   4. Allow to stand until a yellowish sediment of iodoform crystals are formed.
   5. Identify characteristic crystals.

Protein material interferes with the test. Differentiate iodoform crystals from calcium phosphate which may form.

III. Legal Test

1. Add 5cc. urine in test tube.
2. Add 5 drops freshly prepared strong aqueous solution sodium nitroprusside.
3. Add 1cc. potassium hydroxide.
4. Bordeaux red color produced may be due to creatinine, acetone, or paracresol.
5. Differentiate by adding an excess of acetic acid. If acetone is present, the red color will be intensified or change to purple red and finally violet.
   If no acetone is present the color will change to yellow.

IV. Jackson Taylor Test

1. 5cc. urine in test tube.
2. Add 5 drops freshly prepared sodium nitroprusside solution.
3. Layer with concentrated ammonium hydroxide.
4. A purple ring develops indicating presence of acetone.

V. Lange Test

1. 5cc. urine in test tube.
2. Add 2cc. acetic acid.
3. Add few crystals sodium nitroprusside.
4. Shake.
5. Layer with concentrated ammonium hydroxide.
6. A purple ring develops indicating presence of acetone.

This test is very sensitive to diacetic acid. If a purple color develops, check with Gerhard's ferric chloride solution and heat. If the color disappears, acetone is present.

VI. Fitti paldi Test

1. 5cc. urine in a test tube.
2. Add 0.02gm. finely pulverized sodium nitroprusside.
3. Mix until entirely dissolved.
4. Layer with 1cc. ammonia.
5. Allow to stand 15 minutes.
6. A purple ring develops, indicating presence of acetone.
7. Mix. Add acetic acid drop by drop until solution is slightly acid. If the reddish purple tint throughout disappears as the acid is added, there is a large amount of acetone but no diacetic acid present. If the purple blends into a deep ruby red, there is diacetic acid present also.

VII. Rohdenburg Test

1. 5cc. urine in test tube.
2. Add 1 gram ammonium sulfate.
3. Add 7 drops Reagent made up as follows:
   30 gm. ammonium nitrate
   2 gm. sodium nitroprusside
   80 cc. H_2O
4. Layer with 3cc. strong ammonium hydroxide.
5. If acetone is present, a characteristic permanganate color develops slowly. If diacetic acid is present, a very full purple color develops.

VIII. Le Nobel Test

1. 10cc. urine in test tube.
2. Add 5 drops glacial acetic acid.
3. Add 5 drops dilute aqueous solution sodium nitroprusside.
4. Stratify concentrated ammonium hydroxide upon mixture.
5. Violet ring forms at once if acetone is present. Diacetic acid also gives this reaction.

IX. Wallhauser Test

1. 1 drop Scott Wilson reagent on an ordinary microscope slide.
   Reagent:
   18gms. NaOH dissolved in 60cc. water.
   Cool—Add 1gm. mercuric cyanide dissolved in 60cc. H_2O.
   Transfer to a heavy glass jar.
   Add, 0.29gm. silver nitrate dissolved in 40cc. H_2O with constant stirring.
   Will keep 6 months in a tightly stoppered dark bottle.
2. Place over mouth of urine container to form a hanging drop, taking care reagent does not come in contact with container.
3. Allow to stand 2 minutes.
4. Examine macroscopically.
5. If reagent remains clear, the test has given a negative

reaction. If a cloudiness or precipitate forms, a positive reaction is indicated.

X. Rothera Test

1. 10cc. urine in test tube.
2. Add 1 gm. ammonium sulphate.
3. Add 3 drops freshly prepared 5% sodium nitroprusside.
4. Overlay with strong ammonium hydroxide.
   A reddish purple ring at the line of contact is a positive reaction. This test is more sensitive to acetone than to diacetic acid.

XI. Frommer Test

1. 10cc. urine in test tube.
2. Add 3cc. 40% NaOH.
3. Add 10 drops 10% solution salicylaldehyde in absolute alcohol.
4. Warm mixture to about 70° for 5-10 minutes, do not boil.
5. If acetone is present, mixture turns orange, red, reddish purple and dark red. A yellow color is negative. This reaction does not occur with diacetic acid.

Technique and Discussion of a New Test for Acetone

The new reagent is a fine white powder developed by Alexander Galat in the research laboratories of the Denver Chemical Manufacturing Company. The powder is stable, non-caustic, non-poisonous and is composed of sodium carbonate, ammonium sulfate and sodium nitroferricyanide, all in an anhydrous form.

From the point of view of simplicity of technique and safety of use, this new reagent is a marked improvement over the commonly used test tube methods which require several chemicals including strong ammonium hydroxide, sodium nitroprusside, glacial acetic acid or caustic alkali.

The test is very simple to perform. A small amount of powder is placed on a piece of paper in a cone-shaped pile. A drop of urine is placed on the powder and the reaction is complete in approximately one minute. If acetone is present, the powder will turn some shade of purple, the intensity of the color depending on the

amount of acetone present. Thus, a trace will turn the powder a light lavender and a larger amount will turn a dark purple. The color reaction is identical with that found in the violet ring tests and is equally easy to differentiate. When no acetone is present, the powder takes on the color of urine.

This observation is based on 10,000 comparative tests performed on routine urine specimens in the Pathological Laboratory of a general hospital. The standard technique of Lange's chemical test was used as a control. Five drops of a 20% aqueous solution of sodium nitroprusside were used. The new reagent checked perfectly. With positive reactions, a semi-quantitative reading coincided accurately with that of the usual procedures.

In performing these tests, the liquid and dry tests were done independently by two persons on some 5,000 tests and by the same person on the other 5,000 tests. Sometimes a very pale lavender appears in the powder, and fades in about a minute. This is due to the presence of some sulphur compound in the urine. However, as the color is so pale and fades so quickly, it is not mistaken for a positive acetone reaction. The purple color of a positive reaction lasts for some time so that the tests do not have to be read immediately unless desired.

In the course of this work, it has been noted that the test proved equally accurate when performed on a piece of paper, a glass slide, or a slide with depressions such as is recommended for Kline tests. The reactions show equally clearly if one or several drops of urine are added, even though the powder may be completely saturated. No heat is evolved. This is a more rapid way to test for acetone than the usual liquid methods which require ten minutes for the development of a maximum reaction. When a large number of urine specimens are to be examined, the powder is placed in rows and a drop from each urine placed upon the powder. In this manner the results of a large number of specimens may be recorded quickly.

The following substances present in urine in pathological conditions did not give false positive reactions, nor did the deep color of the urines due to their presence prevent the reading of a positive test.

Albumen	Acriflavin
Bile salts	Methylene blue
Blood	Silver nitrate
Creatinine	Crystals
Urobilin	Sulfamids
Urobilinogen	

Another important observation is the economy of this new reagent. The cost of the acetone powder is less than that of the individual chemicals. There is no waste with the powder whereas with the test tube method it is very difficult to avoid using an excess of chemicals. If too much sodium nitroprusside is used, a trace of acetone is not detected due to the dark reddish brown color of the mixture. Another consideration is that, due to the present emergency, some of the chemicals used in the test tube method are expensive and also are becoming difficult to obtain. This new powder is indeed a saving of both time and material.

Summary

1. A short review is given of the tests for the qualitative determination of acetone in urine which are most often found in laboratory manuals and test books.
2. A report is given of 10,000 routine urine specimens examined for acetone by Lange's chemical method and the new Acetone Reagent-Galat, in powder form. The results agreed perfectly.
3. Various substances found in pathological urines did not give false positive or negative results.
4. The new acetone reagent powder is much faster and cheaper to use than the liquid methods and dispenses with any waste of chemicals.

THE KENNY METHOD OF TREATMENT FOR INFANTILE PARALYSIS*

While studies of the means and spread of Infantile Paralysis are carried on and while work is being done to find a chemical cure for this crippling infection, still other workers are striving to improve the methods of treatment.

Much has been written about the Kenny method of treatment for infantile paralysis. Newspapers, magazines and scientific journals all have carried articles dealing with this form of treatment introduced into America by Miss Elizabeth Kenny, the Australian nurse. There still is some confusion as to what is done, why it is done and what the end results are.

To understand how a nurse could present a form of treatment apparently superior to that long advocated by the medical profession calls for a word of explanation about that nurse.

Miss Kenny years ago acquired a truly remarkable knowledge of anatomy and of muscle function. This came as the result of persistent study plus a real interest in things mechanical. Miss Kenny is one of those persons with real mechanical ability. Trained in a different direction, she might well have become a famous inventor. As it is, she has designed several items which are of great service.

Confronted with Epidemic

Before the last war, while serving as a community nurse in a remote part of Australia far removed from all medical help, Miss Kenny was faced with an epidemic of infantile paralysis. She asked for medical advice and assistance. She was told to go ahead on her own and do the best she could as there was no cure. This was a disease with which she was not familiar. She did not know just what to do. She had no definite directions to follow. She therefore examined her patients, looked at them with eyes and a mind trained

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* *From The Importance of Research—Publication No. 42 of The National Foundation for Infantile Paralysis.*

as a nurse but backed by that inherent mechanical ability. Looking at her patients she saw not only children suffering from pain in muscles, but she also saw a series of levers, pulleys and a mechanical system. Something had gone wrong with the mechanism of motion of the human bodies of these people. Arms, legs, hands and feet could not move.

Applied Her Mechanical Talents

To Miss Kenny this was mechanical, not particularly mysterious, and to her there was certainly nothing hopeless about the situation. She saw that muscles were painful and sore, that they were hard, contracted and in spasm, pulling the opposite muscles out of shape so that they could no longer function. What did she do? She applied heat. She tore up old blankets, wrung them nearly dry out of very hot water. She repeated this hour after hour and it relieved the pain. The patients felt much better when the hot packs were applied.

Gradually the pain and spasm relaxed, but arms and legs, hands and feet could not move. Gently, very gently Miss Kenny moved them for her patients, moved them so the patients would not forget how to do so by themselves. With infinite patience she taught them motion. She was equally persistent in preventing muscles from trying to do that for which they were not intended. The paralyzed child in his tremendous effort to regain lost power would bring into play muscles that had no part in the picture, leaving the functionless muscles out of the picture entirely.

Re-Educated Muscles

We all know how this can be done. Watch a man heave and tug, trying to lift a crate far too heavy for him. He will strain with all the muscles of his arms and legs, abdomen and back, and then in addition make the most horrible faces. Contracting the muscles of his face does not help to lift the box. It is nothing more than a surcharge of energy spilling over and accomplishing nothing. So, too, by misdirected nervous impulses will the infantile paralysis patient contract muscles totally unrelated to those that should be moved or which he is attempting to move but which have been knocked out by the disease. The man with his heavy crate will,

after awhile, either succeed in moving it or will cease his efforts. The infantile paralysis patient, however, will keep on trying and unless he is given proper guidance and help, all he may succeed in doing will be to move the wrong muscles. He will be exactly like a man trying to lift the heavy crate by facial contortions alone. A crippled child in this condition presented an abhorrent situation for Kenny the anatomist; it challenged Kenny the mechanical genius, and it certainly was intolerable to Kenny the nurse.

The technique of applying heat to relieve pain and the business of re-educating muscles after the pain and spasm has disappeared was not founded upon any knowledge of physiology or pathology of the disease. But it worked. Miss Kenny found that her patients recovered far better and to a greater extent than had those treated by rigid rest and immobilization under the direction of the surgeons and physicians of her country. Most of her patients recovered, while many of the others after long months in splints and plaster casts remained crippled and deformed.

Her Claims Extensively Tested

In 1940 Miss Kenny came to the National Foundation for Infantile Paralysis, bearing letters of introduction from her Premier in Queensland, Australia. Later she was established at the University of Minnesota, supported in her work entirely by funds from the National Foundation. A program for the study of her results was carried out for the purpose of testing the claims advanced. And she was provided with patients so that the observers could determine if she could produce results that were better than had been previously achieved. Full cooperation was given by the University's departments of Orthopedic Surgery and Physical Therapy.

At the end of the year a special medical committee, appointed by President Basil O'Connor, reported on these results. This report was favorable and, in substance, said that Miss Kenny had apparently been able to do better with her patients than had anyone heretofore. More patients were made available to her and still further study was carried on at the Minneapolis General and the University of Minnesota Hospitals. At the Foundation's Annual Medical Meeting in December, 1941, her work was again reviewed

by our Medical Advisory Committees. To the very best of our ability we have carried out the specific recommendations which were made at that time. They were as follows:

"The Committee on Epidemics and Public Health and the Committee on Education recommend to the National Foundation for Infantile Paralysis that public health officials throughout the nation be given, as promptly as possible, information which may be available regarding the nature of the Kenny technique and its integration with other measures of treatment, and the personnel available for its application in outbreaks of infantile paralysis.

"The Committees recommend furthermore that the training program of the National Foundation for Infantile Paralysis be expanded to provide additional training for considerable numbers of nurses and physical therapy technicians, and recommend expansion of the training program so as to make available additional personnel fully trained in the essentials and principles of the Kenny method.

"The Committees recommend furthermore that the Committee on Medical Publications of the National Foundation for Infantile Paralysis consider immediately the development of a concise manual providing the essential principles and details of the Kenny method and of other applications of hydro therapy and physical treatment in the early stage of infantile paralysis."

Physicians had always been taught that infantile paralysis was really paralysis. Kenny claimed that infantile paralysis was rarely paralysis, but was really spasm of muscles. This was an exactly opposite concept. Physicians used a method of treatment which consisted of immobilization by casts or splints. Kenny contended that these were all wrong for they merely increased the spasm and led to contractures and permanent deformities. According to her, the thing to do was to apply heat to relax the spasm and then start very early passive and active motion followed by re-education of the involved muscles.

Quickly, with aid from the National Foundation, men of science took this problem into their laboratories. Time has not been permitted for evaluation of all points, but certain things have been

brought out showing that Kenny's claims are on a sound foundation.

Spasm Found to Exist

In one laboratory equipped with delicate electrical instruments, the strength of nerve impulses was determined in both paralyzed muscles and those in spasm. As an impulse to produce motion travels down the course of a nerve to a muscle, minute electrical currents are generated. These can be picked up and magnified, as are the waves coming to a radio receiving set. They can be measured, recorded photographically, studied and analyzed. It was found that many muscles which were unable to move were not truly paralyzed in the sense that their nerves were destroyed, but were in spasm. Nerve impulses still were being sent to them, but these did not produce motion.

In the same laboratory, heat was applied after the Kenny manner, to some of these muscles. They showed recovery of their ability to transmit nerve impulses in a normal way and once more to have these impulses translated into useful motion. Other sick muscles were not treated. After long weeks they still showed their spasm! Here was the first real proof that Miss Kenny was treating a condition which actually did exist!

Let it not be misunderstood—there may be true paralysis. In some cases the disease is so severe and the destruction of nerve cells is so complete that there is true paralysis. Nerves are dead. They transmit no impulses to muscles. Neither the Kenny method, nor any other, can restore life to nerves that are dead. Here the damage is permanent. Only surgery and mechanical bracing with steel and leather can provide support and some degree of function in this form of crippling.

Other laboratories carried on studies. In the experimental animal, nerves to muscles were crushed or cut. This is as close as the experimenter can come to producing symptoms like those of infantile paralysis. The muscles supplied by these nerves were paralyzed, but the laboratorian had not killed the nerve cells; he had only temporarily broken the pathway between nerve centers and muscles. Nature repaired this break; nerve fibers regenerated. Some of the experimental animals had their paralyzed legs placed in

splints or plaster casts. Others were allowed to run free, moving about as best they could. The rate and degree of recovery of the two groups were compared. Those with the casts and splints, with complete "rest" of the paralyzed muscles, recovered more slowly and to a lesser degree than those that were not splinted.

While these animals did not have infantile paralysis, and while they were not little children recovering from an acute illness, the two conditions were not too dissimilar. At least it was shown that complete immobilization by mechanical means in such nerve injury cases caused damage. This apparently offered further justification for Miss Kenny's contention that splints and plaster casts should not be used in infantile paralysis.

A great tribute must be paid to Miss Elizabeth Kenny of Australia, not only for her contribution of a new and improved method of treatment for poliomyelitis, but also for giving stimulus to a new line of scientific endeavor and research—research that may go far in supplying information to fill in the many gaps in our knowledge of this disease.

ABSTRACTS

CULTIVATION OF MYCOBACTERIUM TUBERCULOSIS IN A MEDIUM OF MINCED CHICK EMBRYO IN TYRODE'S SOLUTION: M. A. Soltys, Jr. Path. & Bact., vol. LIV, #3, July, '42, p. 375.

Primary cultures of avian, bovine and human types of M. tuberculosis were obtained on the medium described. The avian type medium is prepared as follows:

Fertile hen eggs were incubated 10 days with daily turning. Using sterile tissue culture technique, the chick embryo was ground to a fine pulp in a mortar. Tyrode's solution was sterilized by filtration and placed in 25, 50 and 100 cc. amounts in conical flasks of 500 cc. capacity and the chicken embryo pulp added to 2% concentration.

Cultures were incubated at 37°C. for 7 days. Positive results were obtained as early as 3 days.

STREPTOCOCCUS SALIVARIUS IN INFECTIONS OF THE URINARY TRACT AND ITS SUSCEPTIBILITY TO SULFONAMIDE: E. Neter & M. L. Short, Jr. Urol., vol. 48, #4, Oct., '42, p. 422.

Of 43 specimens of urine from children with urinary infections, 13 showed various types of streptococci while one showed S. salivarius.

Various sulfonamides were added to infusion broth in concentrations of 100 mg.% and to 4.8 cc. of these broths 0.2 cc. of a 1:100 dilution of an 18 hr. broth culture of S. salivarius was added. Growth of this small inoculum was completely prevented by sulfanilamide, sulfapyridine, sulfathiazole, sulfadiazine and sulfaguanidine. When larger numbers of organisms were used, sulfathiazole was found most effective and sulfanilamide least effective of this group.

PHYSIOLOGY OF A BACTERIA-FREE CULTURE OF TRICHOMONAS VAGINALIS. IV. EFFECT OF HYDROGEN ION CONCENTRATION AND OXYGEN TENSION ON POPULATION. G. Johnson, Jr. Parasit., vol. 28, #5, Oct., '42, p. 369.

Difco liver infusion agar slants were overlaid with 6 cc. of modified Ringer's solution containing 5% human serum. The pH of the agar and fluid was adjusted with normal HCl and NaOH and then they were buffered with 0.25% sodium phosphate.

A series of 16 reactions between pH 7.8 and 5.1 was inoculated. Maximum populations were found between pH 5.1 and 6.0.

For the effect of oxygen tension the following medium was used:

Bacto liver infusion	80.0 cc.
Bacto heart infusion powder	25.6 g.
Bacto agar	0.4 g.
Distilled water	720.0 cc.
Normal hydrochloric acid	6.0 cc.

After sterilizing and cooling, 2.0 cc. sterile human serum adjusted to the pH were added to each tube.

Results showed that Trichomonas vaginalis is a facultative aerobe. Oxygen markedly depressed the growth while anaerobic conditions prolonged the period of population increase.

COPPER CONTENT OF COMMERCIAL PEPTONES: H. H. Balch, Jr. Path. & Bact., vol. LIV, #3, July, '42, p. 377.

Reference is made to the work of O'Meara and Macsween (1936) in their investigation of the failure of small inocula of staphylococci to grow in a routine medium and their resulting survey of the copper content of commercial peptones.

The inhibitory agent was not present in freshly prepared media and it could be temporarily removed by heating.

The author found that in general the amounts of copper obtained in his survey of the present were less than those obtained in 1936. This led to a survey for other metals which might have been substituted in the containers used in the preparation of peptone.

Tests for chromium and aluminum were negative. One peptone contained nickel but no evidence could be found that this was

inhibitory.

The author suggests that since some manufacturers have not excluded copper to the degree necessary, the method of preparation of media suggested by Wright in 1933 be used.

SIMPLE METHODS OF STAINING TREPONEMA PALLIDUM: N. E. Goldsworthy & H. K. Ward, Jr. Path. & Bact., vol. LIV, #3, July, '42, p. 382.

The following staining techniques are not claimed to be superior to the darkfield but have been found satisfactory and are more convenient with limited apparatus. Search should be continued at least 10 min. before pronouncing negative.

Victoria blue method:

Grübler's Victoria blue 4R 0.2g. is dissolved in a mixture of 50 cc. methyl alcohol and 50 cc. 5% acetic acid in distilled water. This keeps indefinitely.

The chancre fluid is spread on a clean slide and air dried. 0.5 cc. of stain is used to flood and the slide rocked gently over a pilot flame until the alcohol has evaporated, the point at which the uneven streaming of the stain stops. The stain is washed off with 10% copper sulfate and the slide blotted and examined.

Carbol-gentian violet method:

One volume saturated alcoholic gentian violet is mixed with 9 volumes 5% aqueous solution of phenol. This also keeps well. The fixative is the same as for Fontana, acetic acid 1 cc., formalin 20 cc., and distilled water 100 cc.

Thin smears of the chancre fluid are air dried and covered with fixative 1 min. They are washed under tap and blotted dry. They are covered with stain and allowed to act in the cold 3-4 min. after which a lighted match is held under the slide until steam rises. After ½ minute this is washed off and the slide is dried. The phenol acts as a mordant in this method.

For both methods, fresh material yields much better results than old. The standard Ziehl-Neelsen carbol-fuchsin may be substituted for the carbol-gentian violet in the second technique.

THEORY AND APPLICATION OF THE SERUM "ACID" PHOSPHATASE DETERMINATION IN METASTASIZING PROSTATIC CARCINOMA; EARLY EFFECTS OF CASTRATION: T. J. Sullivan, E. B. Gutman & A. B. Gutman, Jr. Urol., vol. 48, #4, Oct., '42, p. 426.

The method used for "acid" phosphatase was adapted from the King and Armstrong method for alkaline phosphatase. Using 3 units as the normal limit, 85% of a series of 130 cases with roentgenographic evidence of metastases showed high "acid" phosphatase values. Of 70 cases of prostatic carcinoma without roentgenographic findings 89% gave phosphatase values below 3 units per 100 cc. Of 570 patients with non-prostatic disease 90% gave values under 3 units.

The test is recommended as an aid in diagnosis of metastasizing prostatic carcinoma, especially in early cases not yet demonstrable by X-ray.

THE RELATION OF HIPPURIC ACID EXCRETION TO THE VOLUME OF THE URINE: T. E. Machella, J. D. Helm & F. W. Chornock, Jr. Clin. Invest., vol. 21, #6, Nov., '42, p. 763.

A survey of results obtained by the hippuric acid liver function test in 100 cases of evident or suspected hepatic disease showed that the amount of hippuric acid excreted could be influenced by the fluid intake. The greater volumes of urine excreted showed large amounts of hippuric acid.

The retention of bromsulphalein was observed in a higher percentage of cases than was the impaired hippuric acid excretion.

ROLE OF FLUORESCENCE MICROSCOPY IN THE SEARCH FOR TUBERCLE BACILLI: J. M. Kloeck, Proc. Inst. Med. of Chi., vol. 14, #1, Jan. 15, '42, p. 15.

The stain technique recommended is 0.3% auramin in 3% carbolic acid for 4 min. at room temperature. Decolorization is carried out with 0.5% HCl in 10% alcohol for 1 min., followed by drainage and reapplication for 2 min.

The fluorescence equipment is preferable on a monocular microscope though it can be used on a binocular. The advantages of the method are: a larger field, greatest contrast permits faster examination and more acid-fast organisms are stained than in the Ziehl-Neelsen technique.

IMPROVED METHODS OF DIAGNOSIS AND TREATMENT OF INTESTINAL PROTOZOA: A. Knight, Proc. Inst. Med. of Chi., vol. 14, #7, Oct. 15, '42, p. 217.

The stool is emulsified in zinc sulfate solution of specific gravity 1.180 and centrifuged. The cysts float on the surface. This film is lifted with a wire loop and spread on a slide and stained with D'Antoni's iodine and examined.

The disadvantage of the method is that it destroys trophozoites and precysts of E. histolytica so a direct smear is also recommended. The advantages are that a whole specimen can be examined and that the specimen need not be fresh.

THE VALIDITY OF CARIES ACTIVITY TESTS: R. G. Kesel, Proc. Inst. of Med. of Chi., vol. 14, #7, Oct. 15, '42, p. 222.

Three tests are described which gave evidence of being dependable indication of active caries processes.

1. The count of lactobacilli in saliva (Bunting and Jay technique).
2. A mixture of glucose and saliva to which has been added powdered enamel is incubated and agitated 4 hours. The amount of dissolution of the enamel is observed.
3. Wach's method for determining the pH and the total amount of acid formed in a mixture of 3 cc. of saliva and 0.4 cc. of 1% glucose during a 4 hour incubation period.

The results obtained showed fluctuation over a 2 year period and may indicate that the activity of the carious process also fluctuates.

RELATIONSHIP BETWEEN CERTAIN PHYSICO-CHEMICAL CHANGES IN THE CORNIFIED LAYER AND THE ENDOGENOUS BACTERIAL FLORA OF THE SKIN: L. Arnold, Jr. Inves. Derma., vol. 5, #5, Oct., '42.

The cornified layer of the skin resembles a colloidal gel structure. Endogenous bacteria increase or decrease as does the amount of water present. Return to normal requires about half an hour after alkalinization and 2 hours after acidification. The bacteria are increased by alkalinization, exposure to warm water or warm humid air. They are decreased by exposing the skin to acid.

THE QUALITATIVE EXAMINATION OF URINARY CALCULI: J. F. McIntosh & R. W. Salter, Jr. Clin. Inves., vol. 21, #6, Nov., '42, p. 751.

Directions are given for uric acid, xanthine, cystine, carbonate, phosphate, ammonia, calcium oxalate, non-oxalate calcium, magnesium, and for the separation of acid-insoluble material and the microscopic examination.

TREATMENT OF ACNE WITH ORALLY ADMINISTERED ESTROGENS: C. H. Lawrence & N. T. Werthessen, Jr. Clin. Endocrin., vol. 2, #11, Nov., '42, p. 636.

Cyclical manifestations of acne suggested that it might be related to an androgen-estrogen imbalance. Twenty-five cases are reported (14 females and 11 males) who were treated with diethyl stilbestrol or ethinyl estradiol. Fifteen became free from acne in 2-6 months, two more in 8-9 months. The remaining 8 are still under treatment but all show improvement.

NEWS AND ANNOUNCEMENTS

TRANSPARENT MAN COMES TO CLEVELAND

On the eve of its second anniversary civic luncheon, November 20, 1942, Cleveland Health Museum received its first truck loads of exhibit material presented for the duration by the American Museum of Health, New York City. These accessions were selected from the Oberlaender Trust Collection and others displayed in the Hall of Man and Medicine at the New York World's Fair.

The exhibits chosen include such famous pieces as the muscle man, the nerve man, a body book of 16 pages, the nodding head, swinging ear, and acting heart. Models of cell development demonstrate marvels of heredity. More than 300 exhibit items show what is needed to keep the body running, what hazards threaten its existence, and what limitations must be considered affecting its use.

Most widely known is the Transparent Man whose outer form is modeled after the fourth century Greek statute of "The Praying Boy" which was once in the possession of Frederick the Great of Prussia. This exhibit, symbolic of the others which will be given special showings at the Museum in the beginning of 1943, was unveiled by Mrs. Elizabeth S. Prentiss whose support has helped immeasurably to sustain the Museum.

W. W. Peter, M.D., Dr. P.H., associate professor of public health, Yale University, spoke on "Keep the Health Fires Burning." He told how poor health is reducing manpower in this country. One out of five people in the productive age group (20 to 64 years old) are handicapped by chronic diseases, serious mental or physical illness. "Every state and city has its share of these unproductive people," Dr. Peter said. It is important to mention them because "your Museum here and all other contributing agencies can do something to improve conditions."

Bruno Gebhard, M.D., director of Cleveland Health Museum,

who has been instrumental in the designing of New York World's Fair's health exhibits, came to Cleveland in 1940, to design and install exhibit material for its opening and to operate the Museum.

Since its opening, November 13, 1940, it has been visited by more than 66,000 people.

But its influence has been far more widespread than these statements indicate. The attendance figures include people who came from 44 states and several foreign countries, to take the message of Cleveland Health Museum back with them. By means of travelling exhibits the Museum has gone far beyond the territorial limits. It is estimated that more than a half million people from Boston and Bridgeport, to Huron, South Dakota, and Huston, Texas, have seen the Museum's dramatic presentations of health facts.

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District of Columbia

The thirty-first meeting of the District of Columbia Society of Medical Technologists was held at the Army Medical Museum on Thursday evening, October 29th. The speaker was Dr. Allen Eschenbrenner who is in charge of the tissue laboratory of the National Cancer Institute at Bethesda, Maryland. Dr. Eschenbrenner gave a most interesting and informative talk on the organization of the Institute and the wonderful work being carried on. He also extended an invitation to members of the Society to make a tour of the Institute.

Louisiana

The Caddo Parish Society of Medical Technologists was organized in Shreveport, Louisiana, in 1941 and is at present as active as any small professional society can be with the war taking a lot of their spare time and energies. A. Merlin McKinnon is president. There is no state society in Louisiana.

Pennsylvania

The Annual Dinner of the Pennsylvania Society of Medical Technologists and Laboratory Technicians was held at Whitman's,

1626 Chestnut Street, Philadelphia, Pa., on Monday evening, November 9th, 1942, at six-thirty o'clock.

The guest speaker of the occasion was Dr. Max M. Strumia, director of the Bryn Mawr Hospital Laboratories. His subject, a most timely and interesting one, was entitled, "What Research Work Means to Laboratory Work."

We were glad to have Mollie Hill, M.T., ex-president of our branch, the Western Pennsylvania Society of Medical Technologists and Laboratory Technicians, present.

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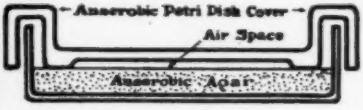

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